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Differences and Similarities between Culture-Confirmed Human Granulocytic Anaplasmosis and Early Lyme Disease

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Lyme disease is transmitted by the bite of certain *Ixodes* ticks, which can also transmit *Anaplasma phagocytophilum*, the cause of human granulocytic anaplasmosis (HGA). Although culture can be used to identify patients infected with *A. phagocytophilum* and is the microbiologic gold standard, few studies have evaluated culture-confirmed patients with HGA. We conducted a prospective study in which blood culture was used to detect HGA infection in patients with a compatible clinical illness. Early Lyme disease was defined by the presence of erythema migrans. The epidemiologic, clinical, and laboratory features of 44 patients with culture-confirmed HGA were compared with those of a convenience sample of 62 patients with early Lyme disease. Coinfected patients were excluded. Patients with HGA had more symptoms ($P = 0.003$) and had a higher body temperature on presentation ($P < 0.001$) than patients with early Lyme disease. HGA patients were also more likely to have a headache, dizziness, myalgias, abdominal pain, anorexia, leukopenia, lymphopenia, thrombocytopenia, or elevated liver enzymes. A direct correlation between the number of symptoms and the duration of illness at time of presentation (rho = 0.389, $P = 0.009$) was observed for HGA patients but not for patients with Lyme disease. In conclusion, although there are overlapping features, culture-confirmed HGA is a more severe illness than early Lyme disease.

Human granulocytic anaplasmosis (HGA) and Lyme disease typically occur in the same geographic areas since both are acquired by the bite of infected *Ixodes* ticks (1–5). The etiologic agents, however, are quite different. HGA is caused by *Anaplasma phagocytophilum*, an obligate intracellular bacterium, whereas Lyme disease is caused by an extracellular spirochetal bacterium, *Borrelia burgdorferi* (1, 2).

The hallmark of early Lyme disease is a distinctive annular erythematous skin lesion, called erythema migrans, that occurs at the site of tick inoculation of *B. burgdorferi*. Erythema migrans usually can be diagnosed clinically without the need for laboratory confirmation (1). In contrast, HGA is not known to be associated with a distinctive clinical feature, and therefore, laboratory testing is required to establish the diagnosis (2–5).

Although *A. phagocytophilum* can be cultured *in vitro*, few laboratories offer such testing. Consequently, most of the reported cases have been diagnosed serologically or through PCR detection of *A. phagocytophilum* DNA. These laboratory methods, while generally reliable, are not necessarily equivalent to the diagnostic gold standard in microbiology of a positive culture. No systematic comparison of culture-confirmed HGA with Lyme disease has been reported. In this study, we compare the epidemiologic, clinical, and laboratory features of culture-confirmed HGA with those of early Lyme disease.

MATERIALS AND METHODS

Study design. This was a prospective, observational study that recorded the baseline epidemiologic, clinical, and laboratory features of patients with culture-confirmed HGA and patients with Lyme disease.

Patients and setting. From 1995 through 2004, patients with potential tick exposure were eligible to enroll when they presented from May through September to the Lyme Disease Diagnostic Center, a walk-in Lyme disease clinic in Valhalla, NY, and (i) had an erythema migrans skin lesion (an expanding erythematous skin lesion ≥5 cm in diameter) or (ii) reported a nonspecific viral-infection-like illness without features suggestive of an upper respiratory tract infection or gastroenteritis. When the walk-in clinic was not in session, patients were referred to the infectious diseases private practice, where they were evaluated and enrolled. Patients hospitalized at the Westchester Medical Center with a febrile illness of undetermined origin who had potential tick exposure and/or erythema migrans also were eligible for enrollment.

At the time of enrollment, prior to antibiotic therapy, a structured interview that included questions about 15 particular symptoms and a physical examination were performed. Laboratory studies included a complete blood count with differential and platelet count, a comprehensive metabolic panel with liver enzymes, and serologic studies to evaluate for HGA. Other diagnostic studies for HGA that were performed at presentation included blood specimens for buffy coat smear to evaluate for morulae, PCR to detect *A. phagocytophilum* DNA in blood, and blood culture for *A. phagocytophilum*. Convalescent HGA serology was performed approximately 1 month later. In the laboratory evaluation of patients with erythema migrans, blood was obtained for culture of *B. burgdorferi* and a 2-mm skin biopsy specimen of the erythema migrans skin lesion was cultured for *B. burgdorferi*.

Subjects provided written informed consent to participate in this study, which was approved by the New York Medical College Institutional Review Board.

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Diagnostic evaluation for HGA. Blood collected in EDTA was used for buffy coat smears, PCR, and culture of *A. phagocytophilum*. Buffy-coat smears were stained with Wright's stain, and 1,000 granulocytes were examined at magnifications of 500 and 1,000 for intragranulocytic morulae. PCR testing was performed on EDTA-anticoagulated whole blood by using a nested reaction with primers HS1/HS6 and HS43/HS45 (6). *A. phagocytophilum* culture was performed as previously described (6, 7). Briefly, 0.2 ml of EDTA-anticoagulated whole blood was inoculated into a flask containing a suspension of 2×10^5 HL-60 cells in RPMI medium containing 10% fetal bovine serum. Cultures were incubated at 37°C under 5% CO_2 for up to 14 days. Every 3 to 4 days, an aliquot of culture was removed to determine the presence of infection of HL-60 cells and to adjust the cell density. Infection of HL-60 cells was confirmed by detecting the characteristic intracytoplasmic inclusions after Wright staining of cytocentrifuged culture aliquots. Antibodies to *A. phagocytophilum* were detected using a local *A. phagocytophilum* isolate with an indirect-immunofluorescence assay that detects IgG, IgA, and IgM, as previously described (8). A titer of ≥1:640 was regarded as suggestive of HGA in this study, since lower titers are less specific and may be found in healthy populations (6). Titers were not assessed above 1:2,560.

Cultures for *B. burgdorferi*. (i) Blood cultures. In EDTA blood collection tubes, three 3-ml plasma samples were obtained. Plasma was separated by centrifugation at 260 × *g* for 15 min. Within 3 h of the time of collection, each 3-ml aliquot of plasma was inoculated into a 70-ml screw-cap plastic flask containing 60 ml of antibiotic-free Barbour-Stoenner-Kelly (BSK) medium, which was prepared as described elsewhere (9, 10). Cultures were incubated at 32°C to 33°C for up to 12 weeks. The cultures were examined by fluorescence microscopy at 2 weeks and thereafter at 2- to 4-week intervals. Sampling for each culture was done as follows. A 10-μl aliquot of culture material was mixed with 10 μl of an acridine orange staining solution (100 μl in phosphate-buffered saline, pH 7.41). Ten microliters of this mixture was placed on a slide overlaid with a coverslip and was examined with a microscope (magnification, ×400). A minimum of 20 high-power fields were viewed for the presence of spirochetes.

(ii) Skin biopsy and culture. Skin biopsy specimens (2 mm in diameter) were obtained from the advancing border of an erythema migrans skin lesion as described elsewhere (11). Biopsy specimens were placed into transport medium, which consisted of modified BSK medium (this preparation of BSK lacks rabbit serum and bovine serum albumin [BSA]) plus rifampin 40 μg/ml, for later laboratory processing. Tissue specimens were then transferred to a microtissue grinder (Fisher Scientific, Pittsburgh, PA), which contained 0.4 ml of modified BSK medium without rifampin, and were ground; 0.2 to 0.3 ml of this suspension was added to a 7-ml screw-cap tube that contained 6 ml of complete BSK medium (with rabbit serum and 35% BSA solution but devoid of antibiotics). The screw-cap tube was tightly capped and incubated at 33°C for the duration of the culturing period. Cultures were examined by means of fluorescence microscopy at 2 weeks and at 2-week intervals thereafter for up to 8 weeks, as previously reported (11).

Exclusion of coinfected patients. Patients with an erythema migrans skin lesion were regarded as having definite or possible HGA coinfection if there was a positive blood culture for *A. phagocytophilum*, a positive PCR assay for *A. phagocytophilum* DNA, a 4-fold rise in antibody titer to *A. phagocytophilum* (regardless of whether the maximum titer reached ≥1:640), or a single positive antibody titer of 1:640. Coinfected patients were excluded from this study. We have previously compared coinfected patients with patients with erythema migrans alone and with culture-confirmed HGA alone (12). Some of the data presented here have been previously reported (12, 13).

Statistical analyses. Categorical variables were assessed using the Pearson chi-square or the Fisher's exact tests for small-sample comparisons. Continuous variables were often skewed and were therefore assessed with the nonparametric Mann-Whitney U test. Spearman rank correlations were computed to assess the degree of association among variables. The 95% confidence intervals (95% CI) were calculated using the exact method. Analyses were done using StataCorp's statistical package Stata. Because of multiple comparisons, a P value of <0.01 was considered to be significant.

RESULTS

The 44 patients with culture-confirmed HGA also had other evidence of active HGA, including a positive buffy coat smear for morulae in 77.3% (95% CI, 64.4 to 90.2) and a positive PCR for A. *phagocytophilum* DNA in 79.6% (95% CI, 67.1 to 92.0). A 4-fold increase in antibody titer to *A. phagocytophilum* to ≥1:640 was found for 32 (78.1% [95% CI, 64.8 to 91.3]) of the 41 patients who had both acute- and convalescent-phase serologies performed. Of the 9 without a 4-fold increase in antibody titer to *A. phagocytophilum* to ≥1:640, 6 showed a titer of ≥1:2,560 on both acute- and convalescent-phase testing (values above 1:2,560 were not determined), 1 showed a titer of 1:1,280 at baseline and a titer of ≥1:2,560 on the convalescent-phase serum sample, 1 showed a titer of <1:80 at baseline and a titer of 1:320 on the convalescent-phase sample, and 1 had a titer of <1:80 on both the acute- and convalescent-phase testing. The culture-confirmed patient with a nonchanging titer of <1:80 had a positive PCR for *A. phagocytophilum* DNA and a positive buffy coat smear for morulae. None of the HGA patients had either erythema migrans on an extensive full-body skin examination or another objective clinical manifestation of Lyme disease, such as 7th nerve palsy, to suggest coinfection with *B. burgdorferi*. In addition, a blood culture for *B. burgdorferi* was negative in the 18 patients with HGA for whom this test was performed.

A convenience sample of 62 patients with erythema migrans, of whom 14 (22.6%) had multiple skin lesions, were included in this study. Forty-four (71.0%) of these patients had a positive culture for *B. burgdorferi* from either a skin biopsy sample or a blood specimen. The 62 patients were randomly selected from 251 patients with erythema migrans without apparent evidence of HGA coinfection, as described previously (12). Specifically, none of the 62 Lyme disease patients had a positive culture for *A. phagocytophilum* or a 4-fold rise in antibody titer to *A. phagocytophilum* between baseline and convalescent-phase testing; in addition, none of the 61 evaluable patients had morulae detected on buffy coat smear examination, and none of the 59 evaluable patients had a positive PCR assay for *A. phagocytophilum* DNA.

HGA patients and patients with early Lyme disease were similar with respect to age and gender (Table 1). Significantly more HGA patients than Lyme disease patients, however, reported a prior episode of Lyme disease (15/44 [34.1%] versus 8/62 [12.9%], $P = 0.009$). There were many indications that patients with HGA were more ill than patients with Lyme disease, although only 2 of the HGA patients were hospitalized (Table 1). Patients with HGA presented after fewer days of illness than patients with Lyme disease ($P < 0.001$), had more symptoms ($P = 0.003$), were both more likely to report a higher body temperature ($P < 0.001$) and to have a higher body temperature on physical examination at the initial visit ($P < 0.001$), and were more likely to have a headache ($P < 0.001$), dizziness ($P < 0.001$), myalgias ($P = 0.004$), abdominal pain ($P = 0.009$), and anorexia ($P = 0.003$). There was no significant difference in the frequency of arthralgias, stiff neck, fatigue, paresthesias, cough, nausea, vomiting, or diarrhea between patients with HGA and those with Lyme disease. A separate analysis comparing HGA with the subset of the 44 culture-con-

TABLE 1 Clinical and epidemiologic features of culture-confirmed HGA versus early Lyme disease (erythema migrans)

Characteristic	No. (%) of patients or other value as indicated		
	Culture-confirmed HGA ($n = 44$)	Lyme disease ($n = 62$)	*P* value
Age (yr [mean ± SD])	51.4 ± 15.3	50.2 ± 14.7	0.269
Male sex	24 (54.5)	30 (48.4)	0.532
History of Lyme disease	15 (34.1)	8 (12.9)	0.009
Tick bite recollection	23 (52.3)	19 (30.7)	0.025
Onset in June, July or August	36 (81.8)	56 (90.3)	0.249
Days of illness to presentation (mean ± SD)	5.3 ± 4.6	9.3 ± 7.5	<0.001
Median (range)	4 (1–22)	7.5 (1–39)	
Total no. of symptoms (mean ± SD)	8.4 ± 3.0	6.3 ± 3.7	0.003
Median (range)	9 (1–14)	6 (1–14)	
Fever, sweats, rigors	39 (88.6)	26 (47.3)	<0.001
Maximum temp (°C) reported	39.2 ($n = 41$)	37.8 ($n = 40$)	<0.001
Temp (°C) at presentation	38.0	37.1	<0.001
Fatigue	37 (84.1)	40 (64.5)	0.026
Headache	36 (81.8)	31 (50.0)	<0.001
Arthralgias	25 (56.8)	38 (61.3)	0.644
Myalgias	33 (75.0)	29 (46.8)	0.004
Stiff neck	20 (45.5)	30 (48.4)	0.766
Anorexia	28 (63.6)	21 (33.9)	0.003
Dizziness	24 (50.6)	15 (24.2)	0.001
Memory impairment	12 (27.3)	17 (27.4)	0.987
Paresthesias	7 (15.9)	17 (27.4)	0.163
Cough	18 (40.9)	13 (21.0)	0.026
Abdominal pain	7 (15.9)	1 (1.6)	0.009
Nausea	14 (31.8)	12 (19.4)	0.142
Vomiting	4 (9.1)	3 (4.8)	0.446
Diarrhea	6 (13.6)	3 (14.8)	0.158

firmed erythema migrans patients showed similar results (data not shown).

Laboratory testing also showed striking differences between patients with HGA and those with Lyme disease (Table 2). The majority of the HGA patients were leukopenic (54.5% had less than 4,500 white blood cells/mm^3), lymphopenic (80% had less than 1,000 lymphocytes/mm^3), or thrombocytopenic (61.4% had less than 150,000 platelets/mm^3), or had an elevated level of the aspartate aminotransferase enzyme (AST) (61.4% had levels of AST greater than 35 U/liter). Thirty-two (72.7%) were either leukopenic or thrombocytopenic. In contrast, only a minority of the Lyme disease patients were leukopenic (6.5%), lymphopenic (16.4%), or thrombocytopenic (9.7%) or had an elevated level of AST (24.2%). There was a direct correlation between the white blood cell count and the platelet count in both the HGA patients (rho = 0.407, $P = 0.008$) and the Lyme disease patients (rho = 0.435, $P = 0.001$) (Table 3).

TABLE 2 Selected laboratory test results of culture-confirmed HGA versus early Lyme disease (erythema migrans)

Characteristic[a]	No. (%) or mean ± SD for patients with:		
	Culture-confirmed HGA ($n = 44$)	Lyme disease ($n = 62$)	*P* value
White blood cell count <4,500/mm^3	24 (54.5)	4 (6.5)	<0.001
Platelet count <150,000/mm^3	27 (61.4)	6 (9.7)	<0.001
White blood cell count <4,500/mm^3 and/or platelet count <150,000/mm^3	32 (72.7)	8 (12.9)	<0.001
Anemia (hematocrit <39% for males and <35% for females)	16 (36.4)	5 (8.1)	<0.001
Lymphocyte count <1,000/mm^3	32/40 (80.0)	10/61 (16.4)	<0.001
White blood cell count ≤3,000/mm^3	11 (25.0)	0	<0.001
Platelet count ≤100,000/mm^3	13 (29.5)	0	<0.001
AST >35 U/liter	27 (61.4)	15 (24.2)	<0.001
ALT >40 U/liter	21/38 (55.3)	14/61 (23.0)	<0.001
Lactate dehydrogenase	404.7 ± 538.3	207.3 ± 58.7	<0.001
Alkaline phosphatase	104.3 ± 55.5	88.5 ± 46.8	0.177
Creatinine	0.92 ± 0.31	0.89 ± 0.19	0.753
Creatine phosphokinase	444.7 ± 1,586.8	103.4 ± 70.2	0.199

[a] AST, aspartate aminotransferase enzyme; ALT, alanine aminotransferase enzyme.

TABLE 3 Correlation between selected variables in patients with culture-confirmed HGA or Lyme disease (erythema migrans)

Variable[a]	Culture-confirmed HGA($n = 44$)		Lyme disease ($n = 62$)	
	Rho	*P* value	Rho	*P* value
Age with				
No. of symptoms	−0.004	0.980	−0.148	0.250
Days of illness to presentation	−0.086	0.580	0.181	0.159
Initial temp	0.276	0.094	0.025	0.845
White blood cell count	−0.095	0.548	−0.287	0.025
Lymphocyte count	−0.240	0.135	−0.290	0.023
Platelet count	−0.143	0.372	−0.334	0.013
AST level	0.150	0.369	0.084	0.519
White blood cell count with				
Platelet count	0.407	0.008	0.435	0.001
Days of illness to presentation	0.087	0.584	0.068	0.604
No. of symptoms	−0.290	0.062	−0.110	0.398
Initial temp	0.010	0.955	−0.012	0.929
AST level	−0.178	0.286	−0.071	0.588
Platelet count with				
Days of illness to presentation	−0.027	0.865	0.398	0.003
Initial temp	0.001	0.994	−0.421	0.001
AST level	−0.286	0.086	−0.138	0.321
No. of symptoms with				
Initial temp	−0.354	0.029	0.245	0.055
Days of illness to presentation	0.389	0.009	−0.098	0.449
AST level	−0.056	0.737	0.299	0.019
Lymphocyte count	0.089	0.586	−0.292	0.022
AST level with				
Days of illness to presentation	0.041	0.808	−0.082	0.528
Initial temp	0.070	0.691	0.064	0.626
Lymphocyte count	−0.121	0.475	−0.288	0.026

[a] AST, aspartate aminotransferase enzyme.

Among HGA patients, there was also a direct correlation between the number of symptoms and the duration of illness at time of presentation (rho = 0.389, $P = 0.009$), which was not found for patients with Lyme disease (Table 3). In both the HGA and Lyme disease groups, there was no significant correlation between the age of the patient and the number of symptoms, initial temperature, white blood cell count, platelet count, or AST level. There also was no significant correlation between the number of symptoms and the initial temperature, the white blood cell count, or the AST level. Platelet counts directly correlated with duration of illness for the Lyme disease group only (rho = 0.398, $P = 0.003$).

In an attempt to look for a way to differentiate HGA patients from Lyme disease patients, we examined selected combinations of variables (Table 4). Thrombocytopenia and/or an elevated AST level showed the greatest difference in prevalence between HGA and Lyme disease: one or the other was present in 93.2% of HGA patients versus 27.4% of Lyme disease patients. Patients with HGA were at least 10 times as likely as those with Lyme disease to have leukopenia with thrombocytopenia, thrombocytopenia with lymphopenia, leukopenia with elevation of the AST level, and

TABLE 4 Selected combinations of variables in patients with culture-confirmed HGA and patients with early Lyme disease (erythema migrans)

Variable[a]	No. (%) of patients with:		*P* value
	Culture-confirmed HGA ($n = 44$)	Lyme disease ($n = 62$)	
White blood cell count <4,500/mm^3 and/or platelet count <150,000/mm^3 (%)	32 (72.7)	8 (12.9)	<0.001
White blood cell count <4,500/mm^3 and platelet count <150,000/mm^3 (%)	19 (43.2)	2 (3.2)	<0.001
White blood cell count ≤3,000/mm^3 and/or platelet count ≤100,000/mm^3 (%)	19 (43.2)	0 (0)	<0.001
Platelets <150,000/mm^3 and/or lymphocytes <1,000/mm^3 (%)	36 (75.0)	13 (21.0)	<0.001
Platelets <150,000/mm^3 and lymphocytes <1,000/mm^3 (%)	23 (52.3)	3 (4.8)	<0.001
White blood cell count <4,500/mm^3 and/or AST >35 U/liter (%)	39 (88.6)	17 (27.4)	<0.001
White blood cell count <4,500/mm^3 and AST >35 U/liter (%)	18 (40.1)	2 (3.2)	<0.001
Platelets <150,000/mm^3 and/or AST >35 U/liter (%)	41 (93.2)	17 (27.4)	<0.001
Platelets <150,000/mm^3 and AST >35 U/liter (%)	19 (43.2)	4 (6.5)	<0.001
Lymphocytes <1,000/mm^3 and/or AST >35 U/liter (%)	41 (93.2)	23 (37.1)	<0.001
Lymphocytes <1,000/mm^3 and AST >35 U/liter (%)	24 (54.5)	2 (3.2)	<0.001
Headache and/or cytopenia (%)	41 (93.2)	35 (56.5)	<0.001
Headache and cytopenia (%)	27 (61.4)	4 (6.5)	<0.001
Headache and/or lymphocytes <1,000/mm^3 (%)	40 (90.1)	35 (56.5)	<0.001
Headache and lymphocytes <1,000/mm^3 (%)	28 (63.6)	6 (9.7)	<0.001
Headache and/or AST >35 U/liter (%)	41 (93.2)	34 (54.8)	<0.001
Headache and AST >35 U/liter (%)	28 (63.6)	6 (9.7)	<0.001
Temperature >38C° on presentation and cytopenia (%)	16 (36.4)	3 (4.8)	<0.001
Temperature >38.9C° during illness and cytopenia (%)	22 (50.0)	5 (8.1)	<0.001

[a] AST, aspartate aminotransferase level; cytopenia, leukopenia <4,500/mm^3 and/or thrombocytopenia <150,000/mm^3.

lymphopenia with elevation of the AST level. Leukocyte counts of ≤3,000/mm^3 and platelet counts of ≤100,000/mm^3 were exclusively found in patients with HGA (43.2% of HGA patients had this level of leukopenia and/or thrombocytopenia versus 0% of Lyme disease patients) (Tables 2 and 4).

DISCUSSION

The findings of our study demonstrate that HGA patients are more symptomatic and are more likely to have cytopenia and abnormal liver function tests than patients with early Lyme disease who have erythema migrans. However, no single clinical or laboratory feature or combination of features could differentiate all of the HGA patients from all of the Lyme disease cases (aside from the presence of an erythema migrans skin lesion, which is an artifact of the study design). Our study was performed at a single medical center and, unlike other studies, used culture to identify patients with HGA. Nevertheless, the clinical and laboratory features of our HGA patients are generally similar to those reported in a meta-analysis of reported HGA cases (2).

To our knowledge, only one other study with at least 20 HGA cases has systematically compared the clinical features of HGA patients and Lyme disease patients. This study, conducted in Wisconsin in 1996 and 1997, compared 83 patients with erythema migrans to 27 patients with HGA; none of the HGA cases was confirmed by culture (14). Eleven (41%) of the HGA patients required hospitalization in that study compared with only 2 in ours. In the Wisconsin study, HGA patients were significantly more likely than Lyme disease patients to have fever, chills, and dyspnea but not headache, fatigue, arthralgias, or myalgias among the 7 clinical variables that were compared. In addition, the leukocyte and platelet counts were significantly lower and the alanine aminotransferase enzyme (ALT) level was significantly higher in HGA patients than in Lyme disease patients.

Our study found that, unlike with Lyme disease, patients with HGA rapidly become more symptomatic over just a few days of illness. This emphasizes the importance of developing laboratory tests or a clinical algorithm to identify cases as quickly as possible. Because our HGA patients met the microbiologic gold standard of culture confirmation, this permits an assessment of the sensitivity of other diagnostic tests. The sensitivity of direct assays, such as the PCR, was 79.6% and that of a buffy coat examination to detect morulae was 77.3%. The sensitivity of acute- and convalescent-phase serologic testing to detect a 4-fold increase in antibody titer to ≥1:640 was 78.1%. Since titers above 1:2,560 were not measured in this study, it is possible that the sensitivity of serology to detect a 4-fold rise to ≥1:640 might have been as high as 95.1% if the endpoint titer had been determined for the 7 patients without a 4-fold rise in titer but whose antibody titer reached 1:2,560. One additional patient had a 4-fold rise in titer to 1:320. Thus, only 1 (2.4%) of the 41 patients who had both acute- and convalescent-phase antibody testing done had persistently low titers (<1:80). Although culture positivity provides unequivocal evidence of active HGA, it is highly probable that, like the other diagnostic tests discussed, culture is also not 100% sensitive.

Particular combinations of other laboratory and clinical parameters were often more sensitive, with results more rapidly available, than the above-described assays for identification of *A. phagocytophilum* infection, with the drawback of a lack of specificity for HGA. For example, over 90% of the HGA patients were found to have thrombocytopenia and/or an elevated AST level (versus 27.4% for Lyme disease patients), lymphopenia and/or an elevated AST level (versus 37.1% for Lyme disease patients), headache and/or either leukopenia or thrombocytopenia (versus 56.5% for Lyme disease patients), headache and/or lymphopenia (versus 56.5% for Lyme disease patients), and headache and/or an elevated AST level (versus 54.8% for Lyme disease patients). Of note, the most severely reduced leukocyte (≤3,000/mm^3) and platelet counts (≤100,000/mm^3) were found only in patients with HGA.

There are several limitations to this study. One is the potential for referral bias in that sicker patients and patients with fever and cytopenia may have been more likely to have been tested for HGA (12). In our related study in which HGA-Lyme disease coinfection was ascertained by routinely testing all patients who presented with erythema migrans for HGA, regardless of symptoms or laboratory test abnormalities, coinfected patients were less likely to report high fever and to have cytopenia than patients with culture-confirmed HGA alone (12).

Another potential limitation is that some of the patients in the HGA alone group may have been coinfected with *B. burgdorferi*. We can state that this group did not have an objective clinical manifestation of Lyme disease such as erythema migrans. We did not attempt to exclude coinfection based on serologic testing for antibodies to *B. burgdorferi* because of prior observations that false-positive IgM antibodies to *B. burgdorferi* may be detected in HGA patients without *B. burgdorferi* infection (15, 16). A similar serologic phenomenon has been observed in a mouse model of *Ehrlichia muris* infection (17). Another limitation of our study is that we made no attempt to assess whether our patients may have been coinfected with babesiosis (18). This omission probably had minimal impact on our findings, however, since babesiosis did not occur in our geographic area of New York until 2001 (19, 20).

In conclusion, although there are overlapping features, culture-confirmed HGA is a more severe illness than early Lyme disease.

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